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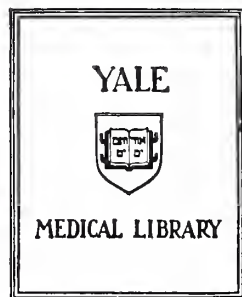


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HEPATITIS B VIRUS AND HEPATITIS A VIRUS  
IN KINGSTON, JAMAICA:  
MATERNAL - CHILD IMMUNITY

JENNIFER LEE HIRSH

1989



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**Hepatitis B Virus and Hepatitis A Virus  
in Kingston, Jamaica:  
Maternal - Child Immunity**

*A Thesis Submitted to the Yale University  
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of the Requirements for the Degree of  
Doctor of Medicine*

by  
Jennifer Lee Hirsh  
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## Table of Contents

Abstract	2
Introduction	3 - 7
Materials and Methods	8 - 12
Results	13 - 15
Discussion	16 - 25
Tables	26
Figures	27 - 28
Appendix	29 - 33
References	34 - 38
Acknowledgement	39



## **Abstract**

**HEPATITIS B VIRUS AND HEPATITIS A VIRUS IN KINGSTON, JAMAICA: MATERNAL-CHILD IMMUNITY.** Jennifer Lee Hirsh and Francis L. Black, Department of Epidemiology and Public Health, Yale University, School of Medicine, New Haven, CT.

This study was performed on women and their infants in Kingston, Jamaica to assess the impact of Hepatitis B Virus and Hepatitis A Virus in this developing country. Serological assays for disease-related antigens and antibodies were performed for epidemiological evaluation. Age-specific prevalences as well as persistence of passive immunity were among the items considered. The prevalence of Hepatitis B Virus antigenemia in the population studied in Kingston, Jamaica, is comparatively low (0.33 percent) with respect to other geographical regions. Analogous findings include a low rate of vertical transmission of Hepatitis B surface Antigen (zero percent), and a low prevalence rate of Antibody to Hepatitis B core in individuals who lack Antibody to Hepatitis B surface Antigen (1.0 percent). Evidence of maternally-derived, passively-acquired immunity to Hepatitis B surface Antigen can be demonstrated as persisting in 40 percent of infants at an average of 6.2 months of age. The overall prevalence of Antibody to Hepatitis A Virus is 74.6 percent, indicating that by child bearing age, at least 75 percent of the population have been infected. The age-specific distribution may either reflect accumulated exposure to Hepatitis A Virus, or may reflect increasing hygienic and socioeconomic standards. Ironically, this may in fact leave the country subject to more apparent hepatitis than some countries where children have been infected with Hepatitis A Virus early in life. Antibodies to Hepatitis A Virus in neonates are maternally-derived, as opposed to having arisen as a response to acute infection. Early infection in homes where the mothers have been infected does not pose a special risk. The prevalence of such passively acquired antibodies to Hepatitis A Virus is high (58.3 percent) and is most likely a result of high initial titers. As a developing country, Jamaica has been able to exact considerable control over infections due to both Hepatitis B Virus and Hepatitis A Virus.



## Introduction

Viral hepatitis is an infection which involves the liver, and four types of etiologic agents have been classified: 1) Hepatitis A Virus (HAV) 2) Hepatitis B Virus (HBV) 3) non-A non-B Hepatitis agents, and 4) the Delta agent, associated concomitantly with HBV. The delta agent is implicated only in the presence of HBV, and as of present, there is no serologic antigenic determinant of non-A non-B Hepatitis. Therefore, in assessing the role of viral hepatitis in Jamaica, only the impact of Hepatitis A and Hepatitis B on women and their infants of the Caribbean island of Jamaica was studied.

### Jamaica

The island of Jamaica encompasses an area of 4,244 square miles. Across it are approximately 2,388,000 inhabitants, 578,000 of whom live in Kingston, the nation's capital. The literacy rate is 86 percent, and the life expectancy 71 years. The economy is supported in part by the bauxite industry, but recently the demand for bauxite has decreased, and tourism has become an increasingly important source of income. Other export products include coffee, sugar, bananas, and, unofficially, marijuana. The per capita income is \$1300.<sup>15</sup>

Although the tourist's perception of Jamaica is that of clear, azure waters off of white sand beaches, the "land of wood and water", as it was called by its first inhabitants, the Arawak, is not all that simple. Fifty percent of the island's population is under the age of twenty, and unemployment remains a large problem.<sup>15</sup> Various "settlement" areas exist within Kingston, where houses are made of concrete and corrugated zinc; "yards" are partitioned by fences made of flattened gasoline cans; and chickens, goats, and children scamper underfoot. These houses have no running water, although water is available at centrally located stations within the settlement. Forty-six percent of the population live in the urban areas, which amount to a mere 5.5 percent of the island's area.<sup>15</sup> The influx of previously rural dwellers has strained the urban public resources. Not all of Kingston, however, is ghetto. The city is home to the dance, theater, and music of Jamaica. The fruits of the land and the wares of the people abound in the marketplace. And Kingston is the location of the University Hospital of the West Indies, which contains major medical facilities as well as the medical school. It is here that this study was undertaken.





## Hepatitis A Virus

Hepatitis A Virus (HAV) is a non-enveloped single-stranded RNA virus which possesses most of the features of a picornavirus, of which enterovirus is a main subdivision.<sup>22</sup> HAV can be inactivated by ultraviolet radiation, exposure to formaldehyde and chlorine, or by boiling for one minute. It appears that all strains of the virus are of one serotype and are antigenically indistinguishable. Antibodies to HAV (Anti-HAV) present early in the disease are of the IgM class; these peak within a few weeks. During convalescence, the IgG class predominates.<sup>41</sup> After acute hepatitis illness, Anti-HAV protects the individual from becoming reinfected.<sup>7</sup> The mode of transmission of HAV is primarily via the fecal-oral route,<sup>51</sup> thus the prevalence of HAV infection is an index of the adequacy of sanitary practices. Although water-borne or food-borne epidemics have been noted, spread of the disease is most often due to person-to-person contact.<sup>51</sup> This occurs as the virus is shed in the fecal material of the infected individual and is transferred to the mouth of another individual, as may occur in conditions of close contact and/or poor hygiene.<sup>24</sup> HAV is transferred from the gastrointestinal tract to the liver, and there, primary replication ensues. The virus is passed into feces via the biliary system.<sup>46</sup> Humans are the only natural epidemiologically important host of HAV.<sup>41</sup> Distribution of the virus is worldwide, based on serological data.<sup>46,51</sup> The age distribution of those infected is related to the level of endemicity of HAV and also the status and rate of change of the socioeconomic, hygienic, and housing conditions of the recent decades.<sup>46</sup> Anti-HAV prevalence has been shown to be related to socioeconomic level, community size, and overcrowded housing conditions.<sup>47</sup> Viral shedding in feces has been demonstrated two to three weeks prior to the onset of illness, as marked by jaundice, and for 8 to 10 days afterward.<sup>24</sup> In developed countries, improvements in sanitation and socio-economic conditions have contributed to a decline in the incidence of HAV.<sup>7</sup> No conclusive evidence for a HAV-carrier state exists,<sup>19,41</sup> but Anti-HAV serves as a serologic marker for previous HAV infection.<sup>7</sup> In the general United States population, the prevalence of Anti-HAV increases with increasing age and decreasing socioeconomic status. In developing countries, HAV infection and the immunity that follows occur predominantly in childhood.<sup>7</sup> In some countries with high HAV prevalence, nearly 100 percent of the individuals become infected and then develop immunity by the age of ten.<sup>24</sup> Perpetuation of HAV is related to population density, level of sanitation and hygiene, and socioeconomic conditions.<sup>46</sup>



## Hepatitis B Virus

Hepatitis B Virus (HBV) is a partly-double stranded DNA virus. The virion, also known as the Dane particle, contains a circular DNA molecule.<sup>57</sup> The virion is comprised of an outer coat and an inner icosahedral nucleocapsid core. Hepatitis B surface Antigen (HBsAg), originally referred to as Australia antigen, is present in the outer coat, and is comprised mainly of two major polypeptides.<sup>7</sup> Various HBsAg subdeterminants have been identified. The common group-reactive antigen, *a*, is shared by all HBsAg isolates. Subtype-specific antigens, *d* or *y*, *w* or *r*, may also be present. The subtype does not affect the clinical course of the disease, but does serve as a marker for epidemiologic studies.<sup>41</sup> Hepatitis B core Antigen (HBcAg) is present on the nucleocapsid core. HBsAg and HBcAg do not cross react. Hepatitis B e Antigen (HBeAg) is found only in HBsAg-positive serum.

The course of HBV infection can be followed by the antigenic markers that appear at various times. HBsAg appears first and is detectable for approximately one to two months after icterus appears. After the clearance of HBsAg, actively acquired antibodies to HBs (Anti-HBs) can be detected, at which time resolution of the disease occurs; however, about 5-15% of infected patients fail to make antibody to HBsAg during convalescence.<sup>41</sup> Anti-HBs continues to persist in most persons after infection and is thought to be the protective antibody.<sup>7</sup> HBsAg has been identified in nearly every bodily fluid, but the most common route of transmission is percutaneous via blood. Other non-percutaneous routes include intimate (especially sexual) contact and perinatal transmission.<sup>20,41</sup>

Hepatitis B core Antigen (HBcAg) is not usually detected in patients with HBV infection because the HBcAg is sequestered within the HBsAg coat.<sup>7</sup> Antibodies against HBcAg (Anti-HBc), however, are detectable from approximately one to two weeks after HBsAg is detected. Anti-HBc is also present weeks to months before Anti-HBs can be detected. Occasionally, there is a temporal gap between the time HBsAg is cleared and the time that Anti-HBs appears. During this time, the only serologic marker of HBV infection may be Anti-HBc.<sup>45</sup> IgG Anti-HBc may remain detectable for many years after infection<sup>27,61</sup> In chronic infection, IgM Anti-HBc titers may indicate the level of virus activity. On the other hand, low titers of only IgG Anti-HBc without other serological evidence of HBV infection may indicate previous Hepatitis B infection.



Hepatitis B e Antigen (HBeAg) is found in temporal association with HBsAg. HBeAg also is associated with high levels of viral replication and represents circulating intact virions, DNA polymerase, and HBV DNA.<sup>7</sup> In self-limited HBV infections, Anti-HBe becomes detectable; this is associated with a lower level of viral infectivity. In protracted infections, HBeAg may persist, marking replicative infection with detectable specific DNA polymerase; this is a time of maximal infectivity. In chronic HBV infection, Anti-HBe is usually detectable whereas HBeAg is not; this disease state is often non-replicative.

### Clinical Course of Acute Hepatitis Infection

Initial prodromal symptoms include anorexia, nausea, vomiting, fatigue, malaise, arthralgias, myalgias, headache, pharyngitis, cough, and coryza. Fever of 100 -102°F may also occur. Once jaundice sets in, the prodromal symptoms usually diminish. The prodrome of Hepatitis B is often more insidious than that of Hepatitis A.<sup>41</sup> The liver becomes enlarged and tender, resulting in right upper quadrant pain and tenderness. Liver function test results show an increase in the serum aminotransferases, SGOT and SGPT, as well as an elevation in bilirubin. These elevations tend to be more prolonged in Hepatitis B.<sup>41</sup> In the case of extensive hepatocellular necrosis, the prothrombin time may become elevated, reflecting a defect in hepatic synthesis of the enzymes of the clotting cascade. Initial neutropenia and lymphopenia precede a relative lymphocytosis. Complete recovery, both clinically and with regards to laboratory test values, usually occurs after one to two months after Hepatitis A and after three to four months in 75 percent of uncomplicated cases of Hepatitis B.<sup>7</sup> Laboratory values may normalize later in the remainder. Acute Hepatitis A is generally a self-limited disease, with fulminant hepatic failure rarely occurring.<sup>49</sup>

However, complicated courses may ensue in those of advanced age or with underlying medical problems, such as diabetes mellitus or congestive heart failure. The case fatality rate in Hepatitis A and B is approximately 0.1 percent, but the fatality rate is one percent in those ill enough to be hospitalized for Hepatitis B.<sup>7</sup> Although HAV has been implicated as the main etiologic agent of fulminant hepatitis in Chilean children,<sup>68</sup> Hepatitis B is responsible for more than fifty percent of fulminant hepatitis, also known as massive hepatic necrosis, in the general population.<sup>7</sup> In this dreaded complication, patients usually present with encephalopathy and may progress to coma. The combination of hepatic failure and encephalopathy may be evidenced by rapidly shrinking liver, elevated prothrombin time and bilirubin levels, ascites, edema,





confusion, and disorientation. Cerebral edema is common. Fatal sequelae include brainstem compression, gastrointestinal bleeding, sepsis, respiratory failure, cardiovascular collapse, and renal failure.<sup>7</sup>

Approximately 5-10 percent of infected patients may never clear HBsAg from their circulation,<sup>23</sup> and are termed HBsAg carriers. There is an increased incidence of HBsAg carriers after HBV infection in neonates, homosexual males, and the immunocompromised individual.<sup>41</sup> HBsAg-positive individuals may 1) be asymptomatic 2) have low-grade chronic persistent hepatitis, or 3) have chronic active hepatitis. HBsAg-carriers also have an increased incidence of cirrhosis and hepatocellular carcinoma (HCC). Evidence for this includes both correlation between geographic distribution of HCC and HBsAg, as well as increased frequency of detection of HBsAg in individuals with HCC.<sup>58</sup> In the United States and Western Europe, hepatocellular carcinoma comprises only 2 to 2.5 percent of malignant tumors discovered at autopsy, yet in parts of Africa and Asia, it may comprise 20 to 40 percent of malignancies.<sup>58</sup> During the course of HBV infection, the viral genome becomes integrated into that of the host. Clones of these hepatocytes are the basis of malignant transformation.<sup>55</sup> The number of persistent carriers in the world, as estimated by seroepidemiological surveys, is now more than 200 million.<sup>23</sup> HCC is a fatal disease, and most patients die within three to six months of gastrointestinal hemorrhage, hepatic failure, or cachexia.<sup>7</sup> Delta hepatitis virus superimposed on chronic Hepatitis B can worsen the severity of the latter, even to the point of development of fulminant hepatitis. HAV does not appear to progress to chronic liver disease.<sup>41</sup>





## Materials and Methods

Maternal serum collection from 304 women was performed at the University Hospital of the West Indies in Kingston, Jamaica between July 11, 1986 and August 18, 1986. These accounted for approximately 85 percent of all deliveries during that time period. A few omissions were because of seriously complicated births, and a few were because the women declined to participate, but most omissions represented problems getting to the patients while they were in the hospital. The women were representative of a broad socio-economic spectrum from all parts of the city and surrounding rural areas; however, they represented a more than usually health-aware population in that a stipulation for admission was initiation of pre-natal care within six weeks of gestation. The ages of the mothers ranged from 16 to 44 years, with a mean of 26.0 years. After written consent was obtained, 10 mL of maternal blood was obtained by venipuncture. The whole blood was allowed to clot. Blood samples were then centrifuged at 1000 rpm for 10 minutes. The serum from each sample was removed with a Pasteur pipette. Serum samples were stored in 5 mL aliquots and kept frozen in a refrigerator freezing unit for approximately three days, until they could be placed in a -40° F freezer. Serum samples were kept frozen and were transported on dry ice from Kingston, Jamaica back to New Haven, Connecticut, where they were stored in a -40°F freezer. On one occasion, serum samples were noted to be thawed between transfer from the best available freezer to the -40° F degree freezer at the University Hospital of the West Indies.

Concomitant placental cord blood samples were obtained, using a 10 mL syringe and 19 gauge needle. When placental cord blood was obtained at night, the blood was refrigerated in a glass Vacutainer™ tube until morning, when it was centrifuged. Centrifugation, serum separation, and storage of placental cord samples were as per the protocol for the maternal samples.

Between December 1986 and January 1987, blood samples from 222 infants of mothers in the study were obtained by venipuncture. The 145 infants not bled were omitted because of a rural address outside the geographic range for home visit (51%), inability to locate the home (31%), failed venipuncture (12%), refusal of permission (4%), or death of the infant (2%). The mean age at the time of venipuncture was 186.6 days. (6.2 months)

In July of 1987, the serum samples were assayed for 1) Hepatitis B surface Antigen (HBsAg) 2) Antibody to Hepatitis B surface Antigen (Anti-HBs) 3) Antibody



to Hepatitis B core Antigen (Anti-HBc) 4) Antibody to Hepatitis A virus (Anti-HAV) and 5) IgM Antibody to Hepatitis A virus (IgM Anti-HAV) in the following manner.

#### Hepatitis B surface Antigen (HBsAg)

All 304 maternal samples were assayed for Hepatitis B surface Antigen. Those maternal samples that were positive for the antigen had assays performed for the detection of antigen in matched cord and infant sera, when available. For detection of Hepatitis B surface Antigen, the Riasure II<sup>TM</sup> radioimmunoassay from Electro-Nucleonics Inc. was used. The test was based on the "sandwich" technique. To each tube with a glass particle containing Antibody to Hepatitis B surface Antigen (goat), 0.2 mL of patient serum was added. Each set of 90 patient serum samples was run with three positive and seven negative controls. Incubation for 1 hour was performed in which 24 cycles of sample agitation and settling on the Riasure<sup>TM</sup> Auto Processor occurred. Any Hepatitis B surface Antigen present in the serum being tested would combine with the Antibody to Hepatitis B surface Antigen (goat). 1.0 mL of 0.01 M phosphate buffer (sodium salts) and 0.15 M NaCl in double distilled water was added to each of the tubes. A single cycle of sample washing (60 seconds agitation and 90 seconds settling) was performed and the contents of each tube aspirated, allowing for 3-4 mm above the glass particle. 0.1 mL of <sup>125</sup>I labelled antibody to Hepatitis B surface Antigen was added to each tube. Incubation for 1 hour was performed, in which 24 cycles of sample agitation and settling occurred. Contents of each tube were aspirated, allowing for 3-4 mm above the glass particle. Four cycles of sample washing (60 seconds agitation and 90 seconds settling) with 1.0 mL of 0.01 M phosphate buffer followed by aspiration were performed. Within 24 hours of completing the assay, radioactivity of each sample was counted in a Packard Auto Gamma 5650 gamma scintillation counter for one minute.

#### Antibody to Hepatitis B surface Antigen (Anti-HBs)

Two-hundred five maternal serum samples, negative for HBsAg, were assayed for antibody to Hepatitis B surface Antigen, using AUSAB<sup>TM</sup> radioimmunoassay from Abbott Laboratories. The samples were selected by criterion of adequate volume of serum but were otherwise felt to be an accurate representation of the population studied. Those maternal samples that were positive for the antibody had subsequent assays performed for the detection of antibody in the infant's serum, when available.



Seven negative and three positive controls were run with each batch of 90 patient serum samples. One polystyrene bead coated with Hepatitis B surface Antigen was added to each well of a reaction tray. 200 microliters of serum and positive and negative controls were added to the respective reaction tray wells. Reaction trays were tapped to ensure complete distribution of liquid over the polystyrene beads. Reaction trays were covered and incubation at room temperature for 18 hours was performed. The contents of the wells were aspirated and each bead was washed in the following manner. A Pasteur pipette attached to a vacuum source was placed next to the bead and the liquid was aspirated from each well. 4 mL of double distilled water was added to the well two times, for a total rinse volume of 8 mL, while simultaneously aspirating the wash solution. Each bead was released from the pipette vacuum at least once during the wash cycle to assure even washing of the bead surface.

At this point, any antibody to Hepatitis B surface Antigen that was present in the patient serum sample, would be bound to the polystyrene bead containing Hepatitis B surface Antigen. 200 microliters of  $^{125}\text{I}$  Hepatitis B surface Antigen was then added to each reaction well. Reaction wells were tapped to ensure that the radioactively labelled antigen solution completely surrounded the polystyrene beads. Reaction trays were covered and incubation for 4 hours at room temperature took place. The liquid solution from each well was aspirated and each polystyrene bead was again washed in the manner previously described. The beads were transferred from their reaction tray wells to assay tubes and were then placed in a Packard Auto Gamma 5650 gamma scintillation counter. Within 24 hours of completing the assay, radioactivity of each sample and control was counted for one minute.

#### Antibody to Hepatitis B core Antigen (Anti-HBc)

Ninety-five maternal serum samples, negative for Anti-HBs, were assayed for the detection of antibody to Hepatitis B core Antigen. These 95 samples were randomly selected except for the one sample known to be HBs-Antigen-positive. The decision not to assay the rest of the samples was based on the results of the first 95, that showed no other Anti-HBc-positivity except in the HBsAg-positive sample. Thus it was unlikely that HBsAg had been cleared, but Anti-HBs had not yet appeared in our sera. The CORAB<sup>TM</sup> assay from Abbott Laboratories was employed. This assay is based on the principle of competitive inhibition, in which non-radioactive antibodies to Hepatitis B Core from serum samples compete with a constant amount of radioactive





antibody to Hepatitis B Core for a limited number of binding sites on beads coated with Hepatitis B core Antigen.

100 microliters of  $^{125}\text{I}$  labelled Antibody to Hepatitis B core Antigen was added to each well of a reaction tray. 100 microliters of patient serum sample or controls (three negative and two positive controls) were then added. The reaction tray was tapped vigorously to ensure thorough mixing of reaction components. One bead containing Hepatitis B core Antigen was then added to each well. The reaction trays were covered and incubated at room temperature for 20 hours. The liquid contents of each well were then aspirated and the beads washed three times with 4 mL of double distilled water for a total rinse volume of 12 mL. The beads were then transferred to assay tubes and the radioactivity counted in a Packard Auto Gamma 5650 gamma scintillation counter for one minute. Controls and unknowns were counted together within 24 hours of completing the assay.

#### Antibody to Hepatitis A Virus (Anti-HAV)

Two-hundred one maternal samples were assayed for antibody to Hepatitis A. The samples were selected by criterion of adequate volume of serum, but were otherwise felt to be an accurate representation of the population studied. Those maternal samples that were positive for the antibody had assays performed on the paired infant's serum, when available. The HAVAB<sup>TM</sup> radioimmunoassay by Abbott Laboratories was employed. This assay is based on the principle of competitive inhibition, in which antibodies to Hepatitis A in the patient's serum compete with radioactively labelled antibodies to Hepatitis A, for Hepatitis A Virus that is coated on a bead.

10 microliters of patient serum sample or control serum (three negative and two positive controls) were placed in the wells of the reaction trays. 200 microliters of  $^{125}\text{I}$  antibody to Hepatitis A Virus was added to each well. One Hepatitis A Virus coated bead was then placed in each well containing a control or serum sample. Each reaction tray was covered and tapped to remove any trapped air bubbles. Reaction trays were incubated at room temperature for 18 hours. The covers were then removed from the trays and the liquid contents of each well were aspirated. Each bead was washed two times with 4 mL of double distilled water for a total rinse volume of 8 mL. The beads were then transferred to counting tubes, and within 24 hours, the radioactivity of each was determined in a Packard Auto Gamma 5650 gamma scintillation counter for one minute.





### IgM Antibody to Hepatitis A Virus (IgM Anti-HAV)

Forty-five infant samples that were positive for antibody to Hepatitis A Virus were assayed for IgM specific for Hepatitis A Virus. These infant samples were selected at random from the 84 Anti-HAV-positive infant samples and were felt to be an accurate representation of the population. The HAVAB-M<sup>TM</sup> assay from Abbott Laboratories was employed. Specimens were diluted 1:200 with 0.15 M normal saline in glass tubes and vortexed gently to ensure mixing. 10 microliters of positive control or negative control or diluted specimen was pipetted into each well. 200 microliters of specimen diluent was added to each well, and trays were tapped to mix contents. One bead, coated with Antibody to Human IgM, was added to each well. Specimens were incubated at room temperature for 2 hours. The liquid was aspirated from each well, and each bead was washed twice with 4.5 mL double distilled water for a total rinse volume of 9 mL. 200 microliters of Hepatitis A Virus (Human) was added to each well containing a bead. Specimens were incubated at room temperature for 22 hours. Liquid was aspirated from each well and again each bead was washed twice with 4.5 mL double distilled water for a total rinse volume of 9.0 mL. 200 microliters Anti-HAV <sup>125</sup>I was pipetted into each well with a bead, and specimens were incubated in a 45° C water bath for 4 hours. Liquid was aspirated and each bead washed with 4.5 mL double distilled water for a total rinse volume of 9.0 mL. Beads were transferred to properly identified counting tubes and immediately placed in a Packard Auto Gamma 5650 gamma scintillation counter. Count rates were determined for one minute.



## Results

### Assay for Hepatitis B surface Antigen (HBsAg)

The radioimmunoassay RIAUSURE™, Electro-Nucleonics Inc., is based on the sandwich technique. If HBsAg is present in the serum sample, it will bind to Antibody to HBsAg (Anti-HBs) coated on the bead placed in each well. In turn, <sup>125</sup>I labelled Antibody to HBsAg (<sup>125</sup>I Anti-HBs) will bind to the HBsAg already bound to the beads, forming an antibody-antigen-antibody "sandwich". (See Figure 1) In this manner, the amount of radioactivity detected will be directly proportional to the HBsAg in any serum sample. The appropriate cutoff value was determined as outlined in the RIAUSURE II™ manual. (See Appendix VI) Only one of the 304 maternal samples tested (0.33%) had a count-per-minute (cpm) value well above that of the cutoff value, and was therefore considered to be positive for Hepatitis B surface Antigen. The mother from whom the sample was taken was 23 years old and lived in a rural area outside of Kingston proper. The matched cord sample had a count-per-minute value that was just above the cutoff value, and was considered to be weakly positive for HBsAg. The matched infant sample, taken at 199 days of age, was negative.

### Assay for Antibody to Hepatitis B surface Antigen (Anti-HBs)

The radioimmunoassay AUSAB™, Abbott Laboratories, is also based on the sandwich technique. Polystyrene beads coated with Hepatitis B surface Antigen (HBsAg) are exposed to serum samples which may contain antibody to Hepatitis B surface Antigen (Anti-HBs). Any Anti-HBs present will bind to the HBsAg on the bead. In turn, <sup>125</sup>I-labelled Hepatitis B surface Antigen (<sup>125</sup>I-HBsAg) is added and will bind to any Anti-HBs already bound to the polystyrene bead, forming an antigen-antibody-antigen "sandwich". (See Figure 2). In this manner, the amount of radioactivity present will be directly proportional to the amount of Anti-HBs in each sample. The appropriate cutoff value was determined as outlined in the AUSAB™ manual. (See Appendix VII). Thirty-three of the 205 maternal samples (16.1%) had cpm values greater than the cutoff value and were therefore considered positive for Anti-HBs. When results were grouped according to maternal age, 4 of 37 (11%) of 16-20 year olds were positive for Anti-HBs; 12 of 73 (16%) of 21-25 year olds; 11 of



60 (18%) of 26-30 year olds; 5 of 31 (16%) of 31-40 year olds were positive for Anti-HBs. (See Table 1) Of these maternal samples that tested positive, twenty of the matched infant sera were tested. Eight of the 20 (40%) infant samples had values greater than the cutoff value and were therefore considered positive for Anti-HBs.

#### Antibody to Hepatitis B core Antigen (Anti-HBc)

The CORAB<sup>TM</sup> assay, Abbott Laboratories, is a competitive radioimmunoassay. Any Anti-HBc present in serum samples competes with a constant amount of <sup>125</sup>I-labelled Anti-HBc for a limited number of binding sites on beads that are coated with Hepatitis B core Antigen (HBcAg). In this manner, the amount of radioactivity detected is inversely proportional to the amount of Anti-HBc in any given serum sample. The appropriate cutoff value was determined as outlined in the CORAB<sup>TM</sup> manual. (See Appendix VIII). Only one of the 95 maternal serum samples had a cpm value that was less than the cutoff value, and was therefore considered to be positive for Anti-HBc. Of note, this was the same sample that was positive for HBsAg, suggesting that the subject may have had an acute concurrent infection.

#### Antibody to Hepatitis A Virus

The HAVAB<sup>TM</sup> radioimmunoassay, Abbott Laboratories, is a competitive binding assay in which any Anti-HAV in a serum sample competes with <sup>125</sup>I-labelled Anti-HAV for binding sites on a bead coated with Hepatitis A Virus (HAV). In this manner, the amount of radioactivity detected will be inversely proportional to the amount of Anti-HAV in any given serum sample. The appropriate cutoff value was determined as outlined in the HAVAB<sup>TM</sup> manual. (See Appendix IX) One-hundred fifty of the 201 (74.6%) maternal serum samples tested had cpm values less than or equal to that of the cutoff value and were therefore considered positive for Anti-HAV. One-hundred forty-four serum samples from infants of mothers positive for Anti-HAV were tested. Eighty-four of those 144 (58.3%) infant sera samples tested positive for Anti-HAV.

When results were grouped according to maternal age, 24 of 33 (73%) of 16-20 year-olds were positive for Anti-HAV; 49 of 76 (64%) of 21-25 year-olds; 47 of 63



(75%) of 26-30 year-olds; 30 of 39 (77%) of 31-40 year-olds were positive for Anti-HAV.

(See Table 2).

### IgM Antibody to Hepatitis A Virus (IgM Anti-HAV)

The HAVAB-M<sup>TM</sup> radioimmunoassay, Abbott Laboratories, is based on the "sandwich" technique. Beads coated with Antibody to Human IgM bind to any available IgM in a given serum sample. Hepatitis A Virus (HAV) is then added; Only if the IgM that is already bound to the bead is directed against HAV, will IgM bind HAV. Next, <sup>125</sup>I Anti-HAV is added and can bind to any HAV already bound in earlier steps. (See Figure 3). In this manner, the amount of radioactivity detected will be directly proportional to the amount of IgM directed specifically against HAV in any given serum sample. The appropriate cutoff value was determined as outlined in the HAVAB-M<sup>TM</sup> manual. (See Appendix X). Forty-five infant samples that were previously found positive for Anti-HAV, were tested for IgM antibody directed against HAV. All (100%) of the forty-five samples were non-reactive (negative) for IgM against HAV.





## Discussion

### Hepatitis B surface Antigen

#### Maternal Sera Hepatitis B surface Antigen:

The Jamaican sera reveal only one of the 304 (0.33 percent) of maternal samples as positive for Hepatitis B surface Antigen (HBsAg). The chronic carrier rate for HBsAg has been estimated as 0.1-0.2 percent in Britain, the United States, and Scandanavia; 3 percent in Greece and Southern Italy; and 10-15 percent in Africa and the Far East.<sup>55</sup> Other estimates of the HBsAg chronic carrier rate are 0.1-0.5 percent in Europe, North America, and Australia; 5 percent in Southern and Eastern Europe and Central and South America; 5-10 percent in the Middle East; and up to 20 percent in some parts of Africa, Asia, and the Pacific.<sup>23</sup> By comparison, the 0.33 percent HBsAg prevalence in Jamaican sera is relatively low. The persistent carrier state has been defined as HBs antigenemia for greater than 6 months.<sup>41</sup> Therefore, this one Jamaican sample that is HBsAg positive may either represent acute Hepatitis or chronic Hepatitis B carriage. Determination would require further serologic marker studies. High titer IgM Anti-HBc would indicate acute Hepatitis B whereas low titer would indicate chronic Hepatitis B. IgG Anti-HBc would also indicate chronic Hepatitis B infection.<sup>55</sup> In any event, it appears that the carrier rate of HBsAg in the population studied in Jamaica is less than or equal to 0.33 percent. In such low HBsAg prevalence areas, inoculation of blood continues to be a major mode of of spread, such as via surgical and dental procedures, intravenous drug abuse, tattooing, acupuncture, and laboratory accidents, as well as communally utilized razors and tooth brushes.<sup>41,69</sup> Others at risk of transmission in low incidence areas include patients on hemodialysis, blood product recipients, residents in institutions for the mentally handicapped, prisoners, male homosexuals, and the sexually promiscuous.<sup>23</sup> These aforementioned risks are most likely the mode of transmission in Jamaica, where the prevalence of Hepatitis B Virus antigenemia is low.

#### Cord and Infant Sera Hepatitis B surface Antigen:

In the Jamaican study, the only umbilical cord sample that was HBsAg-positive, was that matched to the HBsAg-positive maternal sample. The matched infant sample, taken at 199 days (6.6 months) of age, was HBsAg-negative. There is some controversy regarding antigenemia in umbilical cord blood. Some researchers believe that HBsAg can be transmitted across the placenta, but others doubt its true presence



and consider it a result of contamination.<sup>54</sup> It has also been demonstrated that in patients routinely screened during pregnancy, levels of maternal Anti-HBs and cord Anti-HBs are comparable, whereas HBsAg is much less easily detectable in cord blood as compared to maternal blood.<sup>54</sup> It has therefore been postulated that antibody to HBV crosses the placental barrier more easily than does the antigen itself, thus first allowing passive immunization of the fetus in early or mid-gestation and then subsequent prevention of the disease *in utero*.<sup>54</sup> Other studies to evaluate prevention of perinatal transmission have considered infants whose cord blood is HBsAg-positive to have had intra-uterine infection.<sup>34</sup> If we are to believe that HBsAg found in cord blood is not merely a contaminant, then these studies may partially explain why only some of the infants whose cord blood is HBsAg-positive go on to develop Hepatitis B antigenemia: passive immunization provides protection against HBV infection after exposure to maternal HBsAg.

Vertical transmission is defined as the transmission of Hepatitis B Virus from mother to infant.<sup>54</sup> The first observations of "vertical" or "maternal-child" transmission of HBV were made in 1972, when familial clustering of HBsAg was noted.<sup>43</sup> In this project, they studied a group of sisters, all of whom were HBsAg-positive. Among them, they had 24 offspring, 20 of whom were HBsAg-positive (83 percent HBsAg-positivity). Then they studied a group of brothers, all of whom were HBsAg-positive. Among them, they had 7 offspring, only 1 of whom was HBsAg-positive (14 percent HBsAg-positivity). This indicated that maternal-child transmission was the mode of infection. Another fact that supported vertical transmission, was that the one HBsAg-positive child of the brothers, was born to a mother who had Anti-HBs, indicating that she had been previously infected and could have vertically transmitted the HBV infection to her child. Within each family, there was only one subtype of HBV.<sup>43</sup> It has been observed that HBsAg-positive cord blood is not a useful criterion for defining HBV infection in the infant, because infection often could not be confirmed on follow-up serological studies of the child,<sup>57</sup> as in our case. However, the presence of HBsAg in the infant's umbilical cord blood has been shown to be a risk factor in the development of HBsAg-positivity in the infant.<sup>56</sup> In Taiwan, it was observed that 76 percent of infants with HBsAg-positive cord bloods became HBsAg-positive, whereas only 35 percent of infants with HBsAg-negative cord bloods became HBsAg-positive by two months of age.<sup>56</sup> Other related risk factors in the development of infant antigenemia include titer of HBsAg, as determined by complement fixation, and HBsAg-positivity in siblings.<sup>56</sup> Indeed, HBsAg in cord blood is not the only factor influencing vertical transmission of HBV: a study on the maternal-child transmission of



HBsAg in four ethnic groups in Britain revealed similar proportions of HBsAg positivity in the cord bloods of the various groups, but vertical transmission was significantly higher in the Chinese.<sup>18</sup> Other studies have conclusively demonstrated the importance of the maternal Hepatitis B e Antigen in vertical transmission of HBsAg.<sup>4,23,42</sup> Eighty-five percent of infants whose mothers were HBeAg-positive became HBsAg-positive, versus 31 percent of infants whose mothers were HBeAg-negative. The mean length of follow-up time was 12 months for all infants. The presence of maternal HBeAg has also been correlated with maternal HBsAg titer, as determined by complement fixation,<sup>4</sup> and with HBsAg-positivity in the infant's siblings,<sup>4</sup> a factor previously shown to be a risk factor for the development of HBV antigenemia in the infant.<sup>53</sup> A decreasing pattern of perinatal transmission may be seen in areas where improved sanitation and a decrease in number of household members has occurred.<sup>42</sup> Among Japanese women, one of the most important factors is the capability to convert from a HBeAg-positive to a HBeAg-negative state before reaching reproductive age. Because dietary protein has been considered to be of primary importance in immunological competence, it has been postulated that increased protein consumption may be responsible for the decrease in HBeAg-positivity.<sup>42</sup>

Hepatitis B Virus is believed to be causally associated with the development of Hepatocellular Carcinoma (HCC) by the following chain of events: 1) exposure to HBV leads to 2) induction of the HBsAg-carrier state, leads to 3) chronic hepatitis, leads to 4) cirrhosis, leads finally to 5) hepatocellular carcinoma.<sup>58</sup> Evidence to support the causal relationship between HBV and HCC include a close correlation in the geographic distribution of HCC and HBV, particularly with chronic HBs-antigenemia.<sup>58</sup> Also, HBsAg is detectable with unusually high frequency in HCC patients.<sup>58</sup> Between 60 and 90 percent of all HCC patients have concurrent cirrhosis, implying that cirrhosis may be an important factor in the development of HCC.<sup>58</sup> There is convincing evidence that vertical transmission of HBV occurs much more frequently in regions of high- incidence HCC than in low-incidence regions.<sup>4,54,56</sup> This is related to the frequency of HBeAg-positivity in HBsAg-carriers.<sup>4,44</sup> In HCC high-incidence areas, the patients with HCC are on average much younger than HCC patients in low-incidence areas. One possible explanation is that exposure to the etiologic agent occurs earlier in life in the high-incidence areas,<sup>58</sup> such as via maternal-child transmission. Age at which primary HBV infection occurs enhances the probability of becoming a HBsAg-carrier. Ninety-five percent of those infected perinatally become chronic carriers, versus fewer than 10 percent of those infected as adults.<sup>53</sup> Because the mortality of hepatocellular carcinoma is virtually 100 percent and





because in certain countries it is the most common cancer in young men, the impact of HCC is significant, and therefore related factors, such as vertical transmission of the Hepatitis B Virus, are important.

Vertically acquired HBV infection is usually anicteric and is recognized by the appearance of HBsAg between 60 and 120 days after birth.<sup>41</sup> The mechanism of infection is uncertain, although it probably involves ingestion or inadvertent inoculation of maternal blood by the infant during passage through the birth canal.<sup>41</sup> The frequency of vertical transmission of Hepatitis B Virus to infants from HBsAg-carrier mothers varies throughout different parts of the world. Studies in Denmark and Thailand have estimated the HBV vertical transmission rate from HBsAg-positive mothers as zero percent, whereas that in Greece was 6.5 percent, in the United States was 16.5 percent, and in Taiwan was 40 percent.<sup>54</sup> These geographic differences may be attributed to a number of factors, including maternal HBsAg titer,<sup>54</sup> presence of Anti-HBe,<sup>44</sup> and type of early postpartum care and the degree of physical intimacy between mother and infant during the neonatal period.<sup>54</sup> Antigenemia in the infant usually develops by the time the infant is 6 months of age. One study demonstrated that 51 of 63 (81 percent) infants born to HBsAg-positive mothers became HBsAg-positive by 6 months of age.<sup>56</sup> In addition, children who develop antigenemia early on in life, between two and four months, become persistent HBsAg-carriers,<sup>41</sup> and it is known that persistent HBsAg-carriers are at greater risk for hepatocellular carcinoma.<sup>58</sup> In contrast to this, most children who become HBsAg-positive later in life have been shown to lose antigenemia within 6 months of becoming HBsAg-positive.<sup>48</sup>

Our data show that one Jamaican cord blood sample was HBsAg-positive. It is possible that the sample represents intra-uterine infection, with passive immunization of the infant *in utero* and subsequent prevention of HBV infection. However, the count-per-minute rate was just barely above the cutoff value, and a more likely explanation is contamination of the cord sample with maternal serum. The infant born to the HBsAg-positive mother had not developed HBV antigenemia by 199 days (6.6 months) of life. Since maternal-child transmission by HBsAg-positive mothers occurs in 81 percent of infants by six months of age, the Jamaican infant will most likely not develop HBV antigenemia. According to previous studies,<sup>48</sup> even in the event that antigenemia does occur in this 6.6 month old infant, it is not likely to persist. It would appear that the prevalence of Hepatitis B surface Antigen in Jamaica is relatively low, and maternal-child transmission is not a dominant mode of infection.

Antibody to Hepatitis B surface Antigen (Anti-HBs):





In the Jamaican data, 33 of 205 (16.1 percent) of maternal samples were positive for Anti-HBs, indicating that infection had occurred in these individuals. The serologic evidence of current or past infection varies according to geography. The prevalence of Antibody to Hepatitis B has been estimated at 7-10 percent in the United States and Western Europe; 20-50 percent in South America, Southeast Europe, and North Africa; 60-80 percent in Africa and Southeast Asia, and virtually 100 percent in the Pacific Islands.<sup>46</sup> Other estimates of Anti-HBs are 3-5 percent in Switzerland and Belgium; 11 percent in the United States; 15 percent in Israel; 34 percent in Yugoslavia; 62 percent in Senegal; and 78 percent in Taiwan.<sup>60</sup> By comparison, the prevalence of Anti-HBs in the Jamaican population studied is at the lower end of the prevalence spectrum. Hygienic conditions and socioeconomic levels have both been shown to be inversely proportional to the prevalence of HBV infection.<sup>46</sup> Relatively high standards in Jamaica may be partially responsible for the lower Anti-HBs prevalence seen in the Kingston population.

#### Age Distribution of Hepatitis B Infection:

The age distribution for Hepatitis B infection depends on the level of endemicity of the virus in addition to socioeconomic and hygienic standards and cultural factors.<sup>46</sup> Exposure to HBV at an early age is related to degree of intrafamilial contact, socioeconomic and hygienic conditions, and medical standards.<sup>46</sup> These factors all influence the percutaneous inoculation rate of infectious blood and secretions.<sup>46</sup> The exact mechanism of intrafamilial transmission has not been defined.<sup>24</sup>

Studies done in the Pacific Islands show evidence that the prevalence of HBV markers increases with age, peaking at about 40 years of age.<sup>25</sup> In Ethiopia, the prevalence of Anti-HBs was shown to be higher in those over the age of 15 years than in the younger age groups.<sup>65</sup> Papaevangelou reports that prevalence of serologic evidence of past or current infection rises with increasing age throughout life with a rate that depends on the endemicity of HBV infection.<sup>46</sup>

The age distribution of the Jamaican data indicate a rise in prevalence of Anti-HBs with increasing maternal age. (See Figure 4). It appears that a steady prevalence level is reached by approximately 30 years of age, indicating that most infection occurs prior to 30 years. Thus, there is no continuous increase in Anti-HBs prevalence throughout life in this population, signifying a lack of continuous exposure to HBV over the course of a lifetime. This attests to the relatively high socioeconomic and hygienic standards of Kingston, which effectively limit the spread of the disease.



### Anti-HBs: Infant Sera

"Passive Immunization" of the fetus occurs as immunoglobulins of the IgG subclass, antibodies, are transported from the maternal circulation to that of the fetus. These IgG molecules reflect various infectious agents to which the mother has been previously exposed. In the newborn infant at time of birth, levels of immunoglobulin are similar to those of the mother. There is virtually no immunoglobulin of the IgA or IgM subclass because the fetus is protected from antigenic stimulus *in utero*. However, if infection occurs, the fetus will respond with antibody production, mainly of the IgM type. Passively transferred IgG has a half-life of 30 to 40 days, and immunoglobulin concentration decreases with the first few months of life; the lowest levels occur between two and four months of age.<sup>5</sup> This decline is due to both dilution by infant growth, and catabolic degradation.<sup>52</sup> Maternally derived antibodies are considered to be protective against HBV for the first 6 months of an infant's life.<sup>65</sup>

In the Jamaican study, mothers who would be able to transfer Anti-HBs to their infants would need to be Anti-HBs-positive themselves. In this group of women, twenty matched infant samples were available for analysis. Eight of the 20 samples assayed (40 percent) were Anti-HBs-positive at an average of 187 days (6.2 months) of age. This duration of maternally acquired immunity to HBV is similar to that of immunity to measles in Jamaica, as assessed by hemeagglutination-inhibition.<sup>14</sup> Measles antibody half-life in these children was found to be only slightly shorter than that observed in more developed regions.

Given the low HBsAg prevalence rate found in Jamaica, it would seem that the Anti-HBs seen in infants at 6.2 months of age is antibody that is passively acquired from the mother, not antibody synthesized by the infant in response to infection. Therefore, maternally acquired immunity is present in 40 percent of infants at approximately 6.2 months of age. IgM studies in these children would confirm this but were not carried out for reason of cost.

### Antibody to Hepatitis B core (Anti-HBc)

The Jamaican sera that were Anti-HBs-negative were assayed for Anti-HBc. In this manner, assessment could be made of any individuals who had cleared HBsAg but had not yet demonstrated Anti-HBs. Because of the nature of the assay, any class of Antibody to Hepatitis B core, be it IgG or IgM, would be able to compete with radioactively labelled Anti-HBc for binding sites to the HBcAg-coated bead. IgM Anti-HBc indicates the acutely infected, highly infectious patient.<sup>55</sup> Persistent IgM Anti-



HBc indicates ongoing HBV chronic hepatitis.<sup>55</sup> IgG Anti-HBc in the presence of Anti-HBs signals clinical or subclinical HBV infection in the remote past.<sup>55</sup> IgG Anti-HBc in the absence of Anti-HBs indicates persistent viral infection.<sup>55</sup> The CORAB<sup>TM</sup> assay was used as an Anti-HBc survey; further classification of immunoglobulin type could be made if necessary, pending the results of the Anti-HBc survey. The Jamaican sera demonstrated only one sample that was positive for Anti-HBc. This sample was from the same individual who was HBsAg-positive. It is thus most likely that the Anti-HBc detected was of the IgM class of immunoglobulins and represented an acutely infected, highly infectious individual. No other Anti-HBc-positive samples were found in the group of Anti-HBs-negative samples studied, indicating lack of evidence of persistent HBV infection in the population. This is consistent with earlier results showing the low prevalence of HBsAg in the population. The very low prevalence of Anti-HBc in the absence of Anti-HBs, and the low HBsAg prevalence rate, indicate that persistent HBV infection or HBV-carriage is infrequent in the population studied in Jamaica.

#### Antibody to Hepatitis A Virus (Anti-HAV)

##### Maternal Sera:

One hundred-fifty of the 201 (74.6 percent) samples studied were Anti-HAV-positive. The prevalence of Anti-HAV varies with geography. Estimates of Anti-HAV prevalence are 29 percent in Switzerland,<sup>60</sup> 34 percent in Ethiopia,<sup>65</sup> 44 percent in the United States,<sup>60</sup> 76 percent in Senegal,<sup>60</sup> 81 percent in Belgium,<sup>60</sup> 82 percent in Nigeria,<sup>3</sup> 87 percent in Spain,<sup>9</sup> 89 percent in Taiwan,<sup>60</sup> 95 percent in Israel,<sup>60</sup> 97 percent in Yugoslavia,<sup>60</sup> and 100 percent in Namibia/South West Africa<sup>33</sup> and the Pacific Islands.<sup>67</sup> Prevalence of exposure to HAV in various regions of the world is considered to be a function of hygienic and developmental factors,<sup>19</sup> which are of utmost importance in the fecal-oral spread of the disease.<sup>60</sup> Such environmental and socioeconomic conditions, however, are not the only influencing factors. In Belgium, for instance, standards of living are similar to those in Switzerland and the United States; the Anti-HAV prevalence, however, was two to four times that of the other two countries.<sup>60</sup> In general, overall prevalence of Anti-HAV in Jamaica is lower than that of other developing countries and even lower than that of a few developed countries. This low level of Anti-HAV-positivity reflects the efficacy of the Kingston water and sewage systems since the primary mode of transmission of the disease is fecal-oral. As in other studies,<sup>8,9,19,26,31,36,67</sup> the prevalence of Anti-HAV increased with increasing





age. (See Figure 5) Three major age-specific prevalence patterns have been described for Anti-HAV.<sup>24</sup> In the first pattern, rapid acquisition of Anti-HAV occurs early in life, and most individuals become infected by 10 years of age. This pattern is seen in most developing countries, where the spread of enteric viruses is facilitated by the prevailing standards of sanitation and hygiene. The second pattern is that of increasing prevalence of Anti-HAV with increasing age. This pattern occurs in most developed countries and urban populations and demonstrates a steady decline in the incidence of infection over the past three or four decades, as a result of improving standards of sanitation and hygiene. The third age-specific prevalence pattern is relatively rare, in which an epidemic of HAV occurs and the virus spreads until all susceptible individuals have contracted the disease. Having exhausted its supply, the virus then dies out. A high prevalence of Anti-HAV is detected among those individuals alive at the time of the outbreak; no antibody is detected among those born subsequent to it.<sup>24</sup> The age-specific prevalence pattern seen in Jamaica is that of increasing prevalence of Anti-HAV with increasing age, the pattern seen in most developing countries that have experienced recent advances in hygiene and sanitation. This pattern may be interpreted in two ways. First, the increasing prevalence with increasing age could represent continuous exposure to HAV throughout the lifetime of the individual. In this manner, there would be a cumulative effect of Anti-HAV prevalence in the older segments of the population. This interpretation, however, conflicts with the epidemiological data that show this prevalence pattern to occur in regions with recent advances in sanitation and hygiene. Another interpretation of this age-specific prevalence pattern is that the younger segments of the population have a lower Anti-HAV prevalence because of recent improvements in hygiene and sanitation. Before these improvements, an almost universally immune population was achieved at minimal cost by natural HAV infections.<sup>30</sup> Ironically, this situation creates an interesting dilemma. Hepatitis A Virus infection is commonly asymptomatic or at least relatively mild in young children, but as the age of infection increases, so does clinical severity.<sup>41</sup> As the risk of HAV infection continues to decline for younger children, a large susceptible population may result, providing ample opportunity for future epidemics in young adults.<sup>30,41</sup> Therefore although the prevalence patterns of HAV infection reflect improvements in conditions of hygiene and socioeconomic standards in Jamaica, future epidemics in a now-susceptible young adult population are possible.

#### Infant Sera:





One-hundred forty-four samples were available for analysis from infants whose mothers were Anti-HAV-positive. Of these, 84 (58.3 percent) were Anti-HAV-positive. The average age at time of serum collection was 188 days (6.3 months). Although it is known that maternal antibodies protect infants up until 6 to 8 months of age, not all others studies have documented such a high prevalence rate of passively acquired Anti-HAV in infants. One study in Taiwan<sup>30</sup> estimated the prevalence of maternally acquired Anti-HAV as 27 percent at less than one year of age. The estimate for Mexico was 33 percent.<sup>36</sup> The frequency of passively acquired antibody against HAV in Jamaican infants was 58.3 percent. To determine whether these prevalence rates were true indicators of passive immunity or whether they instead represented neonatal infection, infant sera that were Anti-HAV-positive were assayed for Anti-HAV of the IgM subclass. Diagnosis of HAV infection is performed most reliably by determination of IgM-Anti-HAV.<sup>41</sup> If the infant sample were Anti-HAV-positive but not IgM-Anti-HAV-positive, the antibody against HAV would presumably be of maternal origin. The assay for IgM-Anti-HAV revealed that none of the Anti-HAV-positive Jamaican infants tested were positive for IgM-Anti-HAV. Therefore the high prevalence of Anti-HAV seen in the Jamaican study was a reflection of persistent maternally acquired antibody, not acute HAV infection. A similar phenomenon of low prevalence Anti-HBs but high prevalence Anti-HAV in neonates has been reported in Taiwan.<sup>31</sup> The differences in neonatal Anti-HBs and Anti-HAV prevalence are most likely due to a higher titer of maternally derived Anti-HAV.

### Conclusions:

In summary, the prevalence of Hepatitis B Virus antigenemia in the population studied in Kingston, Jamaica, is comparatively low (0.33 percent) with respect to other geographical regions. Analogous findings include a low rate of vertical transmission of Hepatitis B surface Antigen (zero percent), and a low prevalence rate of Antibody to Hepatitis B core in Anti-HBs-negative individuals (1.0 percent). Evidence of maternally-derived passively-acquired immunity to Hepatitis B surface Antigen can be demonstrated as persisting in 40 percent of infants at an average of 6.2 months of age. The overall prevalence of Antibody to Hepatitis A Virus is 74.6 percent, indicating that by child bearing age, at least 75 percent have been infected. The age-specific distribution may either reflect accumulated exposure to Hepatitis A Virus, or may reflect increasing hygienic and socioeconomic standards, which, ironically, may in fact leave the country subject to more apparent hepatitis than some countries where children are



infected with HAV early in life. Antibodies to Hepatitis A Virus in neonates are maternally-derived, as opposed to arising as a response to acute infection. Early infection in homes where mothers have been infected does not pose a special risk. The prevalence of such passively acquired antibodies to Hepatitis A Virus is high (58.3 percent) and is most likely a result of high initial titers. As a developing country, Jamaica has been able to exact considerable control over infections due to both Hepatitis B Virus and Hepatitis A Virus.



**Table No. 1**  
Anti-HBs (Maternal)

<u>Age Range</u>	<u>Age:Weighted Mean</u>	<u>No. (+)</u>	<u>No. Tested</u>	<u>%(+)</u>
16-20 yrs	18.9 yrs	4	37	11
21-25 yrs	23.0 yrs	12	73	16
26-30 yrs	28.2 yrs	11	60	18
31-40 yrs	33.8 yrs	5	31	16

**Table No. 2**  
Anti-HAV (Maternal)

<u>Age Range</u>	<u>Age:Weighted Mean</u>	<u>No. (+)</u>	<u>No. Tested</u>	<u>% (+)</u>
16-20 yrs	18.7 yrs	24	33	73
21-25 yrs	23.1 yrs	49	76	64
26-30 yrs	28.1 yrs	47	63	75
31-40 yrs	33.8 yrs	30	39	77



## Figures

### Figure 1

$^{125}\text{I}$  Anti-HBs  
|  
HBsAg (serum)  
|  
Anti-HBs (bead)

### Figure 2

$^{125}\text{I}$ -HBsAg  
|  
Anti-HBs (serum)  
|  
HBsAg (bead)

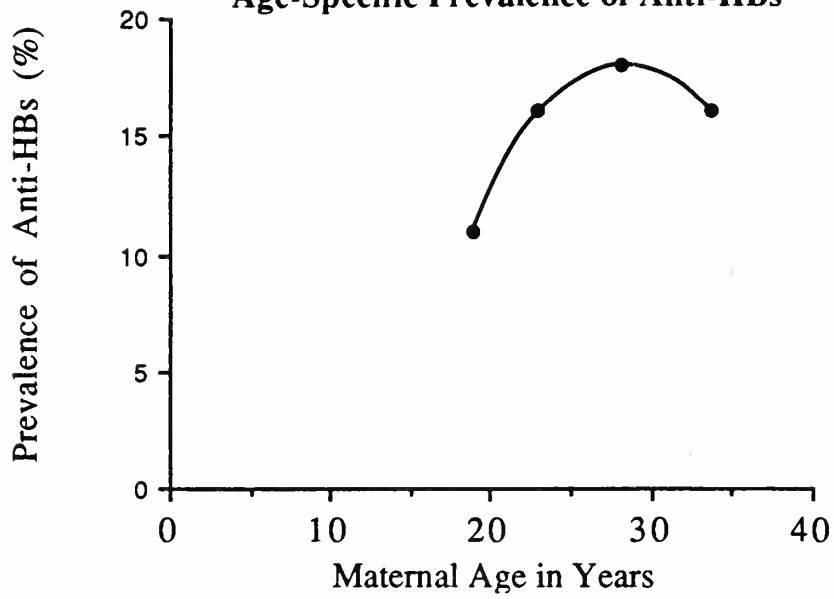
### Figure 3

$^{125}\text{I}$ -Anti-HAV  
|  
HAV  
|  
IgM against HAV  
|  
Anti-Human IgM

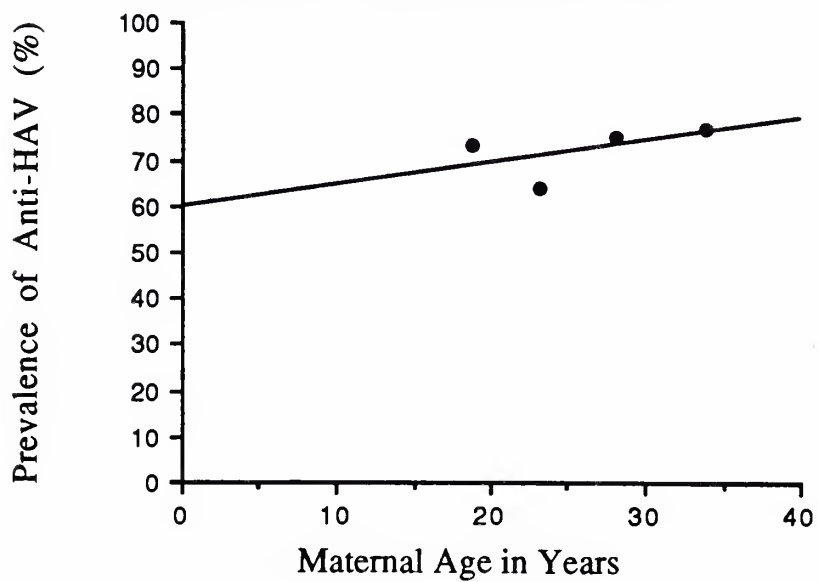




**Figure 4**  
**Age-Specific Prevalence of Anti-HBs**



**Figure 5**  
**Age-Specific Prevalence of Anti-HAV**





## **Appendix**

### **I. Hepatitis B surface Antigen (HBsAg) Lot Nos.**

RIAUSURE II™, Electro-Nucleonics Laboratories, Inc.

Master Lot No. 1419, 1420, 1421

<sup>125</sup>I Anti-HBs: 3901417

CPG Tablet: 3906234

Positive Control: 3903081

Negative Control: 3902115

Buffer: 3410002

### **II. Antibody to Hepatitis B surface Antigen (Anti-HBs) Lot Nos.**

AUSAB™, Abbott Laboratories

Maternal Samples

Hepatitis B surface Antigen beads: 02499M103

Positive Control: 01239BW00

Negative Control: 01495BW03

<sup>125</sup>I Anti-Hbs: 2968M103

Infant Samples

Hepatitis B surface Antigen beads: 03068M303

Positive Control: 02625M302

Negative Control: 03220M303

<sup>125</sup>I Anti-HBs: 05920M103

### **III. Antibody to Hepatitis B core Lot Nos.**

CORAB™, Abbott Laboratories

Master Lot No.: 06435M100

Hepatitis B core Ag (rDNA) coated beads: 02387M202

Positive Control: 03968M103

Negative Control: 02506M302

<sup>125</sup>I Anti-HBc: 05038M203

### **IV. Antibody to Hepatitis A Lot Nos.**

HAVAB™, Abbott Laboratories

Maternal Samples:

Master Lot No.: 06142M100



Hepatitis A beads: 04881M103

Positive Control: 04376M203

Negative Control: 04377M203

<sup>125</sup>I Anti-HAV: 05579M102

Infant Samples:

Master Lot No.: 06769M100

Hepatitis A beads: 04881M103

Positive Control: 04376M203

Negative Control: 04377M203

<sup>125</sup>I Anti-HAV: 05991M202

V. IgM Antibody to Hepatitis A Lot Nos.

HAVAB-M™, Abbott Laboratories

Master Lot No.: 22591M100

Positive Control: 18195M302

Negative Control: 18223M203

Ab to Human IgM coated beads: 18206M302

Specimen diluent: 18176M302

HAV: 19922M203

<sup>125</sup>I Anti-HAV: 21112M202

VI. Calculation of Cutoff Value for Assay for HBsAg

- A. Mean value in counts per minute (cpm) of negative controls calculated by taking the average of the individual negative control values.
- B. Acceptable range for individual values of negative controls is (0.5-1.5) times the mean. All samples must be re-run if more than one result is outside this range.
- C. Mean value (in cpm) of positive controls is calculated by taking the average of the individual positive control values.
- D. Positive Control Mean divided by Negative Control Mean must be greater than 5 for test data to be valid.
- E. Cutoff Value=[Negative Control Mean (cpm)-Background (cpm)] X2 + background (cpm).  
All specimens which are greater than this value must be considered reactive for HBsAg.



## **VII. Calculation of Cutoff Value for Assay for Anti-HBs.**

- A. Negative Control Mean (in cpm) is calculated by taking the average of the individual negative control values.**
- B. Elimination of one (or no) aberrant values of negative controls**
  - 1. Each value must lie within the range of (0.5-1.5) times the negative control mean.**
  - 2. If more than one value lies outside this range, technique problems should be investigated.**
- C. Positive Control Mean (in cpm) is calculated by taking the average of the individual positive control values.**
- D. Positive Control Mean divided by Negative Control Mean must be greater than or equal to 15.0 for technique to be acceptable and data considered valid.**
- E. Cutoff Value = (Negative Control Mean - Background) X 2.1 + Background. Samples with gross count rates greater than this value are considered to be reactive with respect to Anti-HBs.**

## **VIII. Calculation of Cutoff Value for Assay for Anti-HBc**

- A. Negative Control Mean (in cpm) is calculated by subtracting background radiation from individual negative control values and then taking the average of the net values.**
- B. Elimination of one (or no) aberrant values of negative controls:**
  - 1. Each value must lie within the range of (0.5-1.5) times the negative control mean.**
  - 2. If more than one value lies outside this range, technique problems should be investigated.**
  - 3. If one value is outside this range, it is rejected as aberrant, and the negative control mean is recalculated.**
- C. Positive Control Mean (in cpm) is calculated by subtracting the background radiation from individual positive control values and taking the average of those net values.**
- D. Check the validity of each run: Negative Control Mean divided by Positive Control Mean must be greater than 5 to ensure the validity of each run.**
- E. Determination of Cutoff Value:**  
**Cutoff Value = (Negative Control Mean + Positive Control Mean) / 2 + background radiation. Samples with cpm values less than or equal to the**





cutoff value are considered reactive with respect to Anti-HBc by criteria of the CORAB™ test.

IX. Calculation of Cutoff Value for Assay for Anti-HAV.

- A. Negative Control Mean (in cpm) is calculated by subtracting background radiation from individual negative control values and then taking the average of those net values.
- B. Positive Control Mean (in cpm) is calculated by subtracting background radiation from individual positive control values and then taking the average of those net values.
- C. Ensuring the validity of each run: Negative Control Mean divided by Positive Control Mean must be greater than 5 to ensure the validity of each run.
- D. Cutoff Value = (Negative Control Mean + Positive Control Mean)/2 + background. Samples with cpm values which are less than or equal to the cutoff value are considered reactive with respect to Anti-HAV.

X. Calculation of Cutoff Value for Anti-HAV (IgM)

- A. Negative Control Mean (in cpm) is calculated by subtracting background radiation from individual negative control values and then taking the average of those net values.
- B. Positive Control Mean (in cpm) is calculated by subtracting background radiation from individual positive control values and then taking the average of those net values.
- C. Elimination of aberrant values: Individual Positive Control values should lie within the range of (0.5-1.5) times the Mean Positive Control value.
  - 1. If one positive control sample lies outside this range, it is rejected as aberrant and a revised mean positive control value is calculated.
  - 2. If more than one value is consistently outside the range, technique problems should be investigated.
- D. Ensuring the validity of each run: Positive Control Mean divided by Negative Control Mean must be greater than 5.0 to ensure the validity of each run.
- E. Cutoff Value = (Positive Control Mean)/10 + Negative Control Mean + background.



1. Specimens with cpm values less than the cutoff value are non-reactive by criteria of the test.
2. Specimens with cpm values repeatably greater than or equal to the cutoff value are positive by criteria of the test.
3. Specimens within  $\pm 10\%$  of the cutoff value should be retested to confirm initial results.



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